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STUDIES ON THE CHROMOSOMES OF THE COMMON FOWL AS SEEN IN TESTES AND IN EMBRYOS

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Some years ago ('09*b*) I gave an account of the spermatogenesis of the common fowl insofar as I was then able to interpret it. Since then I have spent much time in further observation, partly on the same but mainly on new and better material. Altogether I have been engaged on the problem at intervals for over ten years. In the aggregate this means many months of continuous work inasmuch as it includes summer months as well as those of the school year. I emphasize the element of time, not because time alone is particularly significant, but rather to show that my problem has not been one worked out as a summer's pastime. And while time is not the chief essential in solving problems in avian spermatogenesis, in my estimation such problems will not be solved without the most painstaking and critical study extending over months of protracted daily observation. The cells are small, the chromosomes tend to mass, and fixation is uncertain. This necessitates the preparation of literally hundreds of slides and then the abandonment of the great majority of these in favor of the few which really show adequate detail upon which it is safe to base conclusions. The latter once secured, however, one has material enough in a single slide to occupy many hours and even weeks of the closest scrutiny.

My later studies tend in the main to confirm my earlier observations. Chief among the latter was the finding of a large curved chromosome, comparable to the so-called sex-chromosome of other forms, which typically passes undivided to one pole of the spindle during the division of the primary spermatocyte.

I have found no reason to reverse my opinion on this point since in all of my preparations in which fixation is adequate, I find in abundance a characteristic chromatic element of constant shape and size which behaves in the manner indicated. I have recorded stage readings of over 900 views of it in my preparations and I have seen and studied many others which it was not deemed worth while to record separately. Although the existence of this element has been called in question (Boring and Pearl, '14), I do not see the least reason to doubt its existence or its constancy. However, as my subsequent account will show, and even as suggested in my earlier report, there is strong reason for doubting that it is a single or univalent element. The outcome of my investigation leads me to believe that it is composed of two curved univalent chromosomes which exist separately in the spermatogonial and somatic cells.

The present account is based upon a study of testicular materials from two Langshan, four Plymouth Rock, and two Rhode Island Red fowls, together with sections of a number of embryo chicks of 9, 10, 13, and 19 days of incubation respectively. Of the embryos, chicks of the 10 and 13 day stage were found to be the most satisfactory and consequently were used most extensively. By the tenth day of incubation the sexes can readily be distinguished and from this time on to the thirteenth or fourteenth day there seems to be an unusually plentiful division of primitive germ-cells in progress.

Methods.

Materials were fixed mainly in Gilson's, Flemming's, Hermann's, and Bouin's fluids. The latter, used straight or with various slight modifications, was perhaps the most universally successful fixing agent. When modified, the alteration took the form of reducing the percentage of acetic acid which tends to swell chromosomes and make them agglutinate more than they would do otherwise, or of the addition of chromic acid which in some preparations proved helpful in getting better definition of both chromosomes and cytoplasm.

In the study of the testicular material, smears were used extensively and as a rule proved more satisfactory than sections.

In making the smear a bit of perfectly fresh, warm testis was minced up into a fine pulp by means of a small-bladed scalpel or with fine scissors and then spread into a thin film between two slides in the same manner that a blood film is prepared. The slides after separation were plunged immediately into the fixing agent where they remained from 30 minutes to several hours depending upon the reagent used. At the end of this time they were washed out in the appropriate liquid and all granules or clumps of tissue which might prevent making a very thin, even preparation were picked or scraped away. From this point the slide was treated in the same way that ordinary sections are treated.

While many stains were tried none was found which surpassed iron-hæmatoxylin for Gilson and Bouin material, or saffranin for tissues fixed in Flemming or Hermann. The hæmatoxylin preparations were usually counterstained with orange G, acid fuchsin or Congo red, although the hæmatoxylin alone was found most satisfactory where photography was attempted. Indeed some of my best preparations were found to be practically worthless for photography because of a vivid red or yellow background which I was unable to eliminate by screens and which therefore prevented adequate contrast in the photograph. In preparations stained with safranin a counterstain of Lyon's blue, lichtgrün, or Gentian violet was commonly employed. I find a one per cent. safranin in anilin water a more satisfactory stain than alcoholic solutions of safranin.

While the hæmatoxylin preparations were far better than safranin preparations for photographing, the latter often revealed more detail in the mitotic figures because of the semi-transparency of the chromosomes which often enabled one to see separate elements where only a continuous black opaque mass would be discernible with iron-hæmatoxylin. Delafield's hæmatoxylin gave satisfactory results with spireme and non-mitotic stages but was of secondary value in the study of the fully formed chromosomes. One set of smears stained first in safranin and later in Delafield's hæmatoxylin proved to be unexpectedly helpful in general study, although unfortunately such material did not lend itself at all to photography.

Aceto-carmine, so frequently recommended for fresh tissues, I found of little value with my material. Its only use was to give information quickly about what stages one might expect to find in his other better fixed material. As one might expect, from the considerable amount of acid in this stain, it quickly swells and distorts chromosomes, and as far as my own preparations were concerned it proved untrustworthy. With the tissue of embryos Bouin's fluid, with and without the addition of chromic acid, was used in the main; sections alone were studied.

At the outset, in the naïveté of my inexperience with photography under the microscope, I had hoped to present much of my evidence in the form of photomicrography, but although I made many attempts it speedily became evident that the utility of the photographic camera would be decidedly limited. In order to show the objects of sufficient size, high powers had to be resorted to and with them the plane of focus became so restricted that details which were plainly to be seen with very little manipulation of the fine adjustment were found to be not at all revealed in the photograph.

However, even with this handicap, I feel that my photographs reveal convincing evidence of certain features which I wish to discuss, and I have therefore used them for Plates I. and II. Most of the pictures were taken with a Zeiss 2 mm. apochromatic objective and a number 8 compensating ocular. In a few instances a No. 4 or a projection ocular was employed. A number of different brands of photographic plates were tried but the Cramer contrast plates proved to be the most satisfactory.

General Scheme of Spermatogenesis in the Fowl.

The general histological structure of the testis of the fowl is in the main similar to that of well-known mammalian forms. The chief differences are the more slender character of the seminiferous tubules and the great reduction in the amount of interstitial cells. Thus, the convoluted seminiferous tubules which contain the germinal cells come to make up almost the entire bulk of the testis.

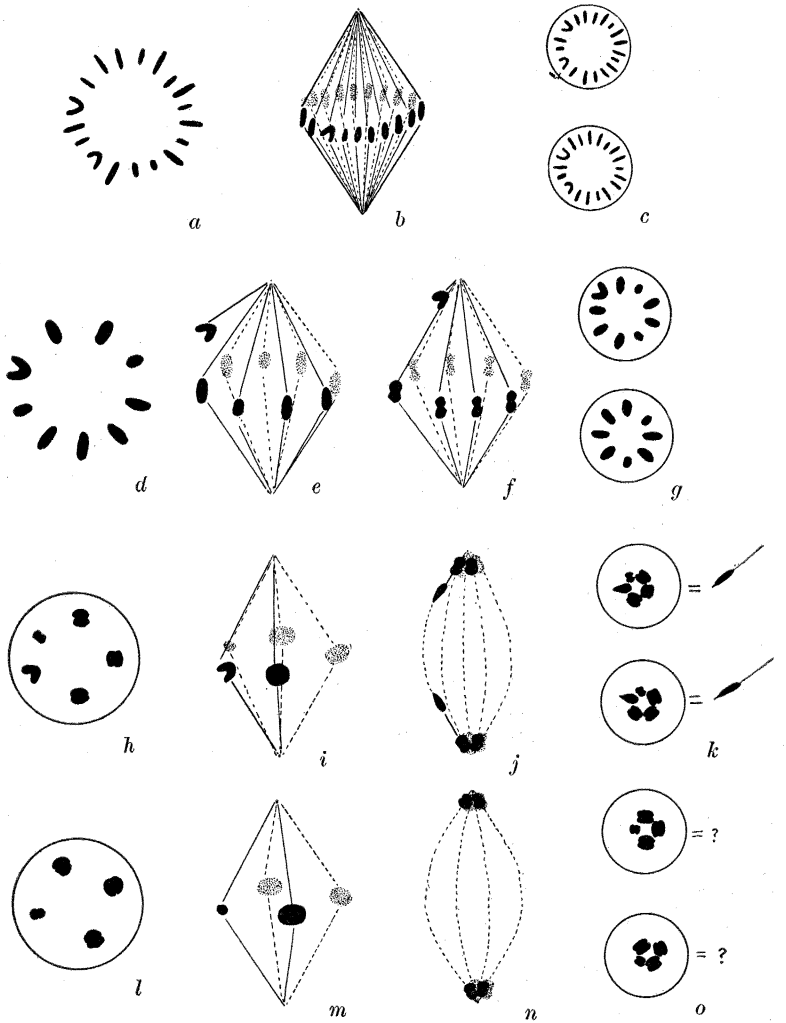
The walls of the tubules are lined by a layer of spermatogonia. These by growth and division give rise to the various later

generations of germ cells which lie inward toward the lumen. As in other well known vertebrates the spermatozoa attach themselves to a Sertoli or nurse-cell for a period before their complete maturation and ejection from the tubule.

The usual four types of cells, (1) spermatogonia, (2) primary spermatocytes, (3) secondary spermatocytes, and (4) spermatids, are present. After a period of spermatogonial divisions, various of the spermatogonia enlarge to become primary spermatocytes which divide to form secondary spermatocytes. The latter divide again to form the spermatids which ultimately transform into spermatozoa. The spermatocytes, both primary and secondary, and the spermatids, seem to be unattached, or at most, to be very loosely attached in the tubule and they therefore readily spill out onto a slide when the tubules are cut. Consequently it is comparatively easy to get an abundance of these cells for smears. On the other hand, it is very difficult to secure spermatogonia in smears in sufficient numbers for purposes of study. They adhere firmly to the tubule wall and even after mincing with scalpel or scissors are seldom found in any considerable quantity.

As seems general in cases in which the germinal cells are arranged in seminiferous tubules, there appear to be proliferating and resting zones in the same tubule, or possibly some entire tubules are quiescent while others are active. This is evinced by the fact that sections or smears from certain regions of the testis show no active mitoses while others exhibit them in varying degrees of abundance. In still other preparations, mainly spermatids, or spiremes of primary spermatocytes, or some other characteristic stage, constitute the main part of the preparation, as if that special part of the testis were in a particular phase of a general wave of spermatogenesis.

A generalized scheme of the spermatogenesis is shown in Text-figure 1. The spermatogonial chromosomes (*a*, *b*), represented in the diagram as sixteen straight, rod-like chromosomes and two curved chromosomes, divide equationally to produce two sets (*c*) of daughter chromosomes. Details regarding the curved chromosomes are given in later pages. Before the next division, which is that of the primary spermatocyte, the usual



TEXT-FIG. 1. Diagram illustrating the general course of spermatogenesis in the common fowl: *a*, polar view of spermatogonial metaphase showing sixteen chromosomes, of which two are characteristically curved; *b*, spermatogonial metaphase viewed from the side; *c*, products of spermatogonial division; *d*, polar view of metaphase in primary spermatocyte showing nine bivalent chromosomes of which one is U-shaped; *e*, *f*, *g*, successive stages in the division of the primary spermatocyte, the curved chromosome passing undivided to one pole and thus producing a dimorphism in the daughter cells; *h*, secondary pairing of the autosomes at the metaphase of the secondary spermatocytes, the curved element remaining unaffected; *i*, *j*, *k*, stages in the division of the five-chromosomed secondary spermatocytes, each spermatid (*k*) receiving five chromosomes, of which four are probably double and therefore equivalent to eight univalent ones; *l*, secondary pairing of the chromosomes in the secondary spermatocytes which did not receive the curved element; *m*, *n*, *o*, stages in the division of the four-chromosomed secondary spermatocytes, the resulting spermatids (*o*) probably not giving rise to mature spermatozoa.

pairing or synapsis transpires so that nine bivalent chromosomes appear in the metaphase (*d*, *e*) of this mitosis. The curved body, so conspicuous an element of this stage, is regarded as a bivalent chromosome formed through the fusion of the two curved chromosomes of the preceding cycle. In the primary spermatocyte, however, it behaves ordinarily as a single element, passing undivided (*e*, *f*) to one pole of the spindle. In this way two classes of secondary spermatocytes (*g*) are formed one with and one without the curved element.

When the secondary spermatocytes are ready for division, typically only four (*l*) or five (*h*) chromosomes appear in the equatorial plate stage. Inasmuch as the chromosomes are of as large size as those of the primary spermatocytes, and since they entered the secondary spermatocytes as groups of 8 or 9 respectively, the condition found at metaphase in the secondary spermatocytes, I regard as a second fusion of the eight ordinary chromosomes by twos thus producing a class of cells which exhibits four and one which shows five chromosomes (4 + the curved element) at the division time. The double nature of these large chromosomes is often indicated by a bilobed condition (*h*, *l*). Their division, however, is not regarded as resulting in a second reduction in the somatic number of the chromosomes. The division seems rather to result in a halving of each element of such a fused pair. It is not uncommon, in fact, for the daughter elements each still to reveal a bilobed condition as they approach the pole (*j*, *n*), or more rarely to resolve partially or wholly into univalent constituents. The curved element lags at the equator of the second spermatocyte while the other chromosomes are diverging toward the poles but it ultimately divides (*j*), a moiety going to each pole. The spermatids (*k*) formed through division of the five-chromosomed spermatocytes are represented in the diagram as forming spermatozoa, those (*o*) descended from the four-chromosomed spermatocytes are indicated as questionable. The evidence on this point is adduced in a later part of the paper.

Spermatogonia and Male Somatic Cells.

The greatest difficulty experienced in the whole course of study was in securing satisfactory preparations of the spermato-

gonial stages, particularly as regards counts and study of individual chromosomes. The chromosomes are small and usually lie in a web of plasma or linin which takes the same dyes the chromosome do themselves. Furthermore, the chromosomes tend so to stick together and so overlie one another as ordinarily to render individual identification uncertain. As far as my preparations are concerned, it would have been impossible to have come to even an approximately accurate conclusion regarding the number and condition of the chromosomes had I depended on what the spermatogonia of adult fowls exhibit. However, the situation may be alleviated in some measure by using the testes of chick embryos, and I have in large measure resorted to the primordial spermatogonia of such material for my more detailed study, supplementing this also by observations on division stages of embryonic somatic cells, particularly those of the renal tubules.

Even under the best of circumstances, it was difficult to find clear cut cases showing all the chromosomes in one section and so disposed as to render an unequivocal count possible. Most of my notes read "over 16" or "not over 18." The difficulty in the main was that sometimes two or more chromosomes overlapped in such a way that it was impossible to say whether the given object should be counted as one or two or possibly three chromosomes. Inasmuch as the chromosomes are not all of the same size, two of the smaller ones closely apposed might easily be mistaken for one of the larger ones. In general in favorable preparations, one could pick out two to four very small ones, two to four relatively large rod-like ones, two strongly curved ones and the rest rod-like ones of intermediate size.

At the outset it should be said that this finding constantly of a pair of curved elements in the male somatic cells came as a surprise to me. I had carried on my observations so long on the large curved element which is so prominent in the primary spermatocytes that my expectation, in studying the cells of embryonic forms, was of finding this same curved element in the somatic cells of the male, or else of not finding it at all since I have always suspected it of being compound in nature. If my mind were prejudiced, it was decidedly in favor of finding a

single, curved element in the male, and a pair of such elements in the female, instead of just the reverse—the actuality of which slowly forced its way upon me as I examined more and more preparations.

Where the chromosomes are sufficiently separated to make one reasonably sure that they were all present without overlapping, I find that I have recorded eighteen, rarely more, as the prevailing number. In other cases, the number visible is set down as 16, or in some few cases fifteen and seventeen; but in the latter cases since all of the smaller ones can not be identified, I have felt that it is a reasonable presumption to suppose that eighteen were there but that one or more of the smaller ones have adhered to or been obscured by some of the others. Thus my counts as recorded in one set of observations run as follows: 123 cells with eighteen chromosomes; 73 cells with sixteen chromosomes visible; 25 with seventeen chromosomes in evidence; 23 cells with fifteen chromosomes in view. Counts can only be made from polar views of equatorial plate stages. Attempts to determine numbers from side views were all futile. To add to the confusion in side views the chromosomes do not all divide at the same time so that some of the daughter chromosomes are well along toward the poles while others are still at or near the equator.

Sometimes instead of the expected number of conventional chromosomes, fewer of the latter are in evidence and the field of the mitotic figure, as seen in polar view, is peppered full of much smaller, deeply staining bodies which appear to be chromatin particles. If one counted each of these a chromosome then the number of chromosomes would sometimes total as many as forty or fifty. I am still at a loss to know whether these are particles of linin or mitochondrial material which take the same dyes the chromosomes do, or whether they are fragmented chromosomes. In favor of the latter view is the fact that occasionally, in lateral views, division figures are to be seen in which these particles lie in the equatorial plate as if ready for individual division.

The particular interest regarding the chromosomes of the spermatogonia centers about the two curved elements which were so frequently in evidence (photos 1–3, Figs. 70–99). They could frequently be picked out even when the other chromosomes

were so massed as to prevent an accurate enumeration beyond observing that other curved ones comparable to the pair in question did not occur. In general, the other chromosomes were smaller and usually appeared as straight, very short or moderately long rods (photo 1, Fig. 72). In some instances a number of chromosomes appeared curved and a particular pair could not be picked out with certainty though such cases were relatively rare (not over 15 per cent. of the cases) when compared with the number in which the typical pair were observable. Many of the cases which at first seemed to be exceptions were, upon careful scrutiny, resolvable into instances in which the ends of two chromosomes swung together, forming a V which at first sight had been mistaken for a single curved element. Fig. 76 shows such an instance; the pair at the right might easily be mistaken for a single chromosome. This mistake is particularly easy to make since the real curved elements frequently show an abrupt bend rather than a long even curve. It is also an error easily fallen into with material stained in iron-hæmatoxylin unless the preparation is strongly decolorized.

As is likely to be true in much cytological material in general, many cells, although in stages of division, were so affected by the reagents or lay in such a position as to render them worthless for accurate observation. These have necessarily been disregarded as it was wholly impossible to affirm that they either bore out or negated the observations made upon more favorable material. Where figures or photos have been made from sections showing only a part of a cell or a part of the chromosomes, as was frequently the case, the preceding and following sections have been carefully inspected to insure as far as possible that the condition intended to be conveyed by the figure is not a misleading one.

Confusion is most likely to arise in late prophase, when the chromosomes have arrived at their rod-like form but have not yet settled down to their final size and position before division. Then, all may show some degree of curvature and one cannot identify with certainty the special pair.

While many observations were made upon the dividing cells of the embryonic testis, as a matter of fact the most satisfactory

details were to be seen in the dividing cells of the nephridial tubules. The chromosomes of this tissue tend less to stick together than do those of the testis and they also stain more sharply. One obvious reason for the greater clearness of equatorial plate stages in such nephridial cells is due to the fact that in dividing to lengthen the tubules, the chromosomes lie at the equator of a spindle which has its longitudinal axis across the shorter diameter of the cell (Fig. 104). This shortening of the spindle produces a proportionally greater equatorial spread with the result that the chromosomes are spaced further apart. The legends accompanying the respective photos and figures state whether the picture in question is of a somatic or of a germ cell.

Photos 1 to 3 and Figs. 70 to 99 show representative conditions in various somatic and spermatogonial cells. Most of them speak for themselves. In such figures as 86, 87 and 98 the pairs of special elements appear as of inordinate size. This is due in part to their actually greater size but also to the fact that the ends of most of the other chromosomes have been cut away in the preceding and following sections.

Photo 3 is of a spermatogonium of an adult cock. While the ordinary chromosomes were clumped so that little detail could be determined beyond the fact that there were no long curved elements among them, the two special elements lay well to one side and were particularly easy to identify. As already mentioned the spermatogonia of adult fowls were much less satisfactory to study in detail than were those of embryonic chicks. However, various other spermatogonia in adults, showing the two curved chromosomes, were found as were also a number in the guinea-fowl, and hosts of them were discovered with two special projecting elements which one suspects of being the same curved elements as are visible in spermatogonia and somatic cells when conditions can be clearly seen, but concerning which one is not absolutely sure. In any event such conditions do not negate the evidence found in the more favorable cells.

The relative positions of the two elements usually in evidence were, for example, approximately those indicated in such cells as photos 1 and 2 and Figs. 71, 72 and 76. That is, when seen in

polar view, they lay most commonly with their ends toward the periphery of the equatorial plate and their plane of curvature at right angles to the chief axis of the spindle, and frequently they were relatively near together, being separated by only some two to four small chromosomes. Sometimes, however, the two curved elements lay at opposite sides of the spindle as in Fig. 86. Rarely the position of the two bodies on the spindle was such that one had its plane of curvature parallel to the long axis of the spindle.

The Chromosomes of the Female.

Before continuing with the later phases of spermatogenesis it will be well to consider the condition of the chromosomes in the early germ and tissue cells of the female chick. Photos 4 to 7 and Figs. 100 to 117 show representative stages of the chromosomes in the primordial oögonial divisions and in the divisions of somatic cells as seen in the nephridial tubules. The latter, as in the male, were often the more favorable for study. The divisions of the primordial oögonia, indeed, were considerably more difficult to decipher than those of the spermatogonia because for some reason, possibly correlated with the larger size of the cell, the chromosomes were frequently longer and more thread-like and consequently more likely to interlace and otherwise alter in fixation. Although in the ten-day chick whole nests of oögonia would be found in various stages of division it was rare to find stages that one could make heads or tails of when it came to studying individual chromosomes. It was hopeless to try to do anything with the late prophase stages because the chromosomes even up to entering the equatorial plate remained decidedly elongated and nearly all of them showed more or less curvature. However, not a few dividing oögonia were found in such condition as to show that a single characteristic element was commonly present. While several chromosomes in a given equatorial plate might show more or less curvature, this special element, commonly larger than the others, was usually discernible in such division figures as showed the chromosomes in any condition beyond that of a confused mass. In somatic cells, especially those of growing uriniferous tubules, conditions were considerably better. The individual chromo-

somes were not so long and the special, unpaired curved element was frequently in evidence. While in some cases I have recorded the occurrence of more than one curved element in individual cells, these additional curved chromosomes were usually smaller than the element in question and readily distinguished from it. In the first hundred polar views of dividing cells showing any understandable detail, recorded from one preparation of a ten-day female chick, for instance, I find that 43 have a single unmistakable large curved element, 20 have a long element which is probably the curved element with the curve turned directly away from or toward the observer, and therefore out of perspective, 27 have a number of the chromosomes curved so that it is impossible to pick out any special one as the particular element in question, and 10 do not show what could positively be identified as a special curved body, the doubt arising as to whether what appeared to be curved element was not two chromosomes with the ends overlapping.

Photos 4 to 7 and Figs. 100 to 117 show characteristic appearances of the cells of females as revealed by the photographic camera, or the camera lucida. For the details regarding each figure the reader is referred to the legend which accompanies it. Fig. 115 represents a type found occasionally in which while an actual curved element was not to be found, an individual chromosome much longer than its mates was to be seen and is probably to be interpreted as the special element in question. Not infrequently it held the hæmatoxylin stain much less tenaciously than did the ordinary chromosomes, becoming yellowish brown in color, while they remained black. Fig. 110 shows this lighter staining element as unmistakably curved. Photos 6 and 7 are side views of equatorial plate stages. Each shows a special elongated chromosome at one edge of the chromosomal plate. While these elements do not appear in the photograph to be curved as a matter of fact a slight shift of focus of the microscope reveals a decided curvature in each. Although in many cases the flat surface of the curved element was turned at right angles to the long axis of the spindle (Figs. 107, 110), not infrequently it lay parallel to the latter as in Fig. 108 or even at an acute angle.

Fig. 111 shows a late metaphase in which the special element is dividing lengthwise. Figs. 112, 114 and 117 illustrate various cases in which division is in progress or has occurred.

From time to time an especially bothersome element appeared in some cells which complicated the interpretation of conditions in the tissue of females. It took the form of an elongated rod which occurred along with the curved element. Like the curved element, it often was of lighter staining capacity and had the former not been distinctly visible as a separate body the second element might have been mistaken for it. Whether or not it consists of several of the ordinary chromosomes which have remained attached and are dividing as one body is not wholly clear. This seems to be the most plausible interpretation although it is not apparent why such a mass should stain less deeply. That such compounding does occur occasionally in the cells of various vertebrates, I am strongly inclined to believe, from the evidence I have seen of variations in the number and size of chromosomes in other avian and mammalian tissues. Apparently chromosomes in such cases represent congeries of units of a lesser order which may be done up in fewer and larger, or more numerous and smaller packets, contingent upon as yet unknown conditions of equilibria in the cells. This probability constantly hangs over the student of these forms as the chief one which is likely to vitiate his conclusions. All the corrective he has is to make great numbers of observations and base his conclusions upon the conditions which he finds strongly preponderant.

Figs. 101 and 102 are drawings made from dividing cells in a five-day chick. While sex cannot be determined from macroscopic evidence, or even microscopic examination of the indifferent gonads at this early date, from the fact that the cells each contain but a single large curved element the inference would be that the embryo bearing these cells is a female. In this connection, the writer was greatly interested in Fig. 2 of a recent paper by Swift ('15). The figure shows a section through the indifferent gonad of a four-day chick embryo and includes a division figure of a primordial germ-cell. Although Swift's study was not on chromosomes and he does not mention the

condition of the chromosomes of this particular cell, he has unwittingly, and therefore without even subconscious bias, given us what appears to be a beautiful example of a single curved or U-shaped chromosome among a number of straight rod-like ones. Unfortunately the cell is so cut that a few of the chromosomes lie in another section so that the evidence is not absolutely convincing although since nearly all of the chromosomes are shown, it appears to be decidedly corroborative of my own findings.

Primary Spermatocytes.

The later spermatogonia, in the resting condition, very commonly show two chromatin nucleolar-like bodies which, judging from the fact that one or both often display a decided curvature, are possibly to be identified with the two curved elements of the division stage. In some cases the resemblance is decidedly clear, in others less so. Sometimes the bodies appear to be spherical or oblong rather than curved, but this appearance is due in some instances at least to the position they occupy with reference to the observer. Fig. 118 shows a group of such spermatogonial nuclei as seen in a section of the testis. Parts of the nucleolar-like bodies have been cut away in some but in the nucleus below and to the left, one element is present in its entirety and so oriented as to show its curvature. Later when the growth period begins these bodies tend to fade out although in such stages as Fig. 119 they are still visible. The disappearance seems to be in the main a loss of staining capacity (Fig. 121) rather than an actual dissolution.

During the period of growth and development in which the products of the spermatogonial divisions become primary spermatocytes although important activities are obviously in progress in the nucleus, I have been unable to determine sufficient constancy in the details on which to base an adequate conclusion regarding such important processes as synapsis. There is the usual increase in nuclear size and with it characteristic appearances at certain stages. For example, there is an apparently early stage in which the nucleus seems to have the chromatin material scattered through it in the form of a fine dust, with occasional fine strands of linin and small fragments of chromatin

in evidence (Figs. 119, 120). Then follow stages in which the stainable content seems confined to a very fine spireme with the chromatin practically all concentrated in the filaments (Fig. 121). In especially favorable preparations it can be seen to be strung along as a string of very small round chromomeres. This stage is evidently the leptotene stage of modern literature. Whether the threads are separate and as numerous as the diploid chromosomes or whether they constitute a continuous or a discontinuous spireme could not be determined. It can only be averred that the strands are much finer, and more numerous than those which exist just prior to the appearance of the individual chromosomes. Any number of instances of two of the fine filaments lying parallel one to another could be cited, but whether such threads constitute true pairs in process of parasynapsis or whether the condition is purely incidental could not be determined. Regarding the question of parasynapsis I can only affirm that the evidence is certainly not against such an interpretation and as far as it indicates anything it rather points toward parasynapsis than otherwise.

Consequent upon the leptotene stages come the contraction phase or synizesis in which the filaments condense into what appears to be an indiscriminate tangle (Fig. 122), and then slowly follows a second extension of the chromatic filaments throughout the nuclear area until the nucleus is occupied once more by a spireme, this time of fewer and coarser filaments (Figs. 123, 124). In Fig. 123, two elongated, nucleolar-like elements are visible.

The spireme in its various phases is a stage of considerable duration if one may infer from its universal presence in almost any section taken from an active testis. When it once starts to condense into the individual chromosomes the operation, judging by the scarcity of stages to be found, is a comparatively rapid one. Just how the bivalent chromosomes form from the spireme I have not been able to discover despite much time spent in the attempt to do so. One finds a relative abundance of metaphase stages but in every case the chromosomes, when identifiable, are well established, relatively compact bodies. The most diligent search has failed to reveal the accessory chromosome or chromo-

somes conspicuously laid down in advance as nucleolar-like bodies, after the manner recorded as so characteristic in insects. Spiremes and synizetic stages may be found occasionally which show one or more chromatin condensations which might be interpreted as nucleoli but they rarely show anything in size or shape which could lead one to identify them with the curved elements of the spermatogonia or of the larger special element found later in the spermatocytes.

Figs. 125 to 132, in which all details have been depicted as accurately as possible, show representative views of an interesting condition which exists just prior to the formation of the chromosomes in the primary spermatocytes. The spireme of such a stage as that shown in Fig. 124 seems gradually (Fig. 125) to break up into a series of characteristic smaller and larger chain-like groups. In some of these it is difficult to decide whether each formation consists of a series of transparent, bead-like bodies encased in a thin shell of deeply staining material, or whether it arises through the twisting of two filaments one about the other. The latter is certainly the condition in some cases, particularly of the smaller elements where the free ends of the threads are distinctly visible, as for instance in Fig. 130, and I am inclined to think that it also prevails in the other instances. The formations thus established condense gradually into bodies of smaller size, certain ones of which, at least, take on the appearance that is so characteristic of some types of tetrads (Fig. 132). Even in metaphase, when the chromosomes of the primary spermatocytes are well established, a tetrad-like condition of individual chromosomes may occasionally be detected in strongly decolorized preparations. In some instances, indeed, one member of such four-groups apparently becomes displaced and divides as a smaller, independent member, thus confusing the chromosome count. Occasionally this seems to occur in more than one member of a given set of chromosomes so that two or more of such quarter-sized individuals may appear at the formation of the equatorial plate stage.

As noted in my earlier paper ('09*b*) there are frequent nuclear divisions without a corresponding division of the surrounding cytoplasmic mass. Commonly from two to four large primary

spermatocyte nuclei may be seen in process of growth or of division in a common mass of cytoplasm which shows no indication of being divided into separate cells. The condition often persists through the following division stages with the result that from eight to twelve spermatid nuclei may be found in one syncytial mass. More frequently, however, what appears to be a fragmentation of the cytoplasm without the appearance of definite walls, into clumps containing fewer nuclei occurs. Looked at from the standpoint of the relative rôles of nucleus and cytoplasm in heredity, this establishment of specific nuclei in a more general matrix of cytoplasm might be regarded perhaps as indicating the more individual nature of the former and more generalized constitution of the latter. On the other hand, inasmuch as but little cytoplasm enters into the makeup of the ultimate spermatozoön the condition may not be of as much significance as one is at first thought tempted to attribute to it.

Notwithstanding the scarcity of intelligible prophase stages in the primary spermatocytes, at metakinesis, there is to be found in abundance a characteristic chromatic element of constant shape and size which behaves like the typical X-element of insects. Whatever the theoretical interpretation may be, the presence of this element can be abundantly demonstrated. It not infrequently comes to be at or near one pole of the spindle while the ordinary bivalent chromosomes are still in the equatorial plate stage (photos 8–13; Figs. 134, 137, 145, 146, 149). At a slightly earlier period it is nearer the equatorial plate—commonly just above or below (Photos 15, 20, 22; Figs. 135, 138, 139, 159), but very frequently also at one edge (Figs. 148, 154–158). Unless preparations are very strongly destained it is likely to escape detection in such positions as the last, since in heavily stained preparations the whole chromatic figure becomes a blurred mass. In fifteen cases I find I have recorded curved elements at each pole of the same spindle, the presumption being in such instances that the original special chromosome has divided as do the ordinary bivalent ones of the spermatocyte.

Boring and Pearl ('14) have published a paper on some phases of the spermatogenesis of the domestic chicken. They find

little or no evidence of such an element as I have described beyond what might charitably be regarded as a mere accident. Although they say that "it is impossible to count chromosomes accurately in this material," and that to attempt to work out a continuous detailed history of spermatogenesis in the Barred Plymouth Rock "would require a large amount of imagination" they have not hesitated to label a number of their mass-effects as containing "no possible X." While they assert that some 15 per cent. of their preparations show a possible X-like element, 85 per cent. do not. It is only a fair question to ask if this 85 per cent. shows anything else, and if the pictures they give are a fair sample of the whole, I think any unbiased observer will have to admit that they do not.

I agree with them in finding that it is very difficult to get a satisfactory count of chromosomes except in the most favorable material. But even without an absolutely accurate count it can be determined in many preparations of chicken testis that there is one peculiar curved or bean-shaped element present unlike the others which are round or oblong, and above all, an element that behaves in a characteristic way. When such observations can be strengthened by further unequivocal cases where a count is obtainable, then it seems to me that legitimate conclusions can be drawn regarding what is the usual occurrence in the material in question. The evidence as seen under the microscope undoubtedly shows that there are sometimes such fusions of the ordinary chromosomes as to reduce the count—and this is, as I have already maintained, a constant factor in secondary spermatocytes—but this by no means invalidates the evidence regarding a special X-like element, inasmuch as fused ordinary chromosomes would not frequently be mistaken for it.

In doing cytological work I have always accepted as a working maxim that preparations which show nothing definite must be ignored and only those taken into account which have sufficient distinctness to be capable of a reasonable interpretation. Almost any cytological preparation I have ever seen—and I have examined preparations of some of the best technicians in America and Europe—will show a number of dividing cells, let us say, which can not be said to give evidence of anything much beyond

the fact that the cell is in process of division. To determine detail regarding a particular chromosome, one naturally has to ignore such preparations and choose only those cells which either show evidence for or against the point at issue. There is always a residue of cells which must fall in the neutral zone of non-significance, and this residue of course becomes increasingly great when one is dealing with inherently unfavorable material, as the germ-cells of the rooster undeniably are. Even in as classical and clear cut an object as *Ascaris* with its small number of chromosomes, I find that a large percentage of the cells showing chromosomes give no clear cut evidence of the conditions which we universally teach as characteristic of *Ascaris*. On the other hand they do not negate these teachings.

Regarding the X-like element of the male fowl I found that it exists in great abundance. Up to date I have in my notes 963 unequivocal cases recorded in primary spermatocytes, to say nothing of many other cases I have seen but not specifically noted down. In the field under the microscope from which such photos as 9 and 34 have been taken, under slightly lower power some 3 to 5 other division figures each showing such a curved element may be seen. The element in question when seen from the side is always in the form of a curved rod, usually thick and plump looking, though occasionally more slender and proportionately longer. Photo 8 is a good clear-cut example of how it appears in a favorable preparation. Inasmuch as it may lie in almost any conceivable position with reference to the pole of the spindle, or the point of view of the observer, obviously the majority of views of it will be at some other angle than that depicted in photo 8 and correspondingly difficult to represent by photography. Indeed, for every one to be found in a suitable position to photograph, many could be positively identified as the same element, but lying in such a plane that some shift of the fine adjustment of the microscope was necessary to see the whole chromosome. Often tangentially lying elements or those with the curved surface turned directly toward the observer require careful focusing to determine the exact shape and dimensions of the object.

Photos 12, 14, 23 and 27, when examined under the microscope

where slight shifts of focus are possible, are just as clear cut cases as photo 8, although because of foreshortening of the object as seen in one plane, not one of them gives as convincing a photograph.

Figs. 152–158 and photos 30–32 are polar views showing the X-like body lying at the edge of the equatorial plate. If such a cell were being viewed from the side instead of the pole it is obvious that the special element would in all likelihood be indetectable and such a view would probably, by one who desired to make a case against the existence of such a body, be recorded as evidence against it. Such side views must of course occur, but in even many of these a careful scrutiny of the mitotic figure shows that it is asymmetrical, extending out further on one side from the spindle than on the other. In such instances it is as legitimate to infer that one has in the chromatic band before him a special element related to the other chromosomes as in Fig. 157, as it is to consider it in any other way. Or in any event it can not be legitimately recorded as evidence against the existence of such a body. It is obvious further that if such an element as shown in Fig. 157 lay directly back of or in front of the other chromosomes as viewed by the observer, instead of at the side, the equatorial plate would then appear symmetrical and such a stage, though having the element in question, would be likely to be recorded as without it.

Photo 33 shows a telophase of a dividing primary spermatocyte in which the undivided X-element lies close to one set of the divided chromosomes after the latter have migrated to their respective poles. The opposite pole has no such body. Photos 34 and 35 show somewhat similar conditions, only in these cases matters are complicated by the beginning of the secondary fusion of chromosomes which is so characteristic a procedure preliminary to the next division. In such cases, of which a number have been observed, the X-like element is apparently tied to the rest of the adjacent chromatin mass by two heavy linin fibers (Figs. 151, 164, photo 34). It is possible that this is determined by the fact that the curved chromosome is in reality probably double in nature. Figs. 150, 151 and 164 are camera-lucida drawings of such conditions showing details that could not be revealed by photography.

In my earlier paper ('09*b*), based upon a much more meager series of preparations and on the whole considerably less satisfactory from the standpoint of technique, I made mention of a third chromosome which was at times associated with the curved one. A more extensive survey of material shows this to be of much less frequent occurrence than I then thought it to be, and probably of no special significance. Such a smaller chromosome may infrequently be seen either toward one pole along with the X-like body or alone, or even toward the opposite pole. So far as I can analyze the condition it is merely one division product of one of the smaller chromosomes of the equatorial plate which, following a precocious division, has passed on in advance of the other chromosomes toward one pole. My earlier drawings also give the impression of considerable irregularity in the outline of the chromosome there designated as the odd. While such irregularities may be found I must conclude from my later material that the appearance is due in the main to unsatisfactory fixation as my recent, better preparations all show what, when one considers the difficulty of the material to be handled, is a surprising uniformity in the appearance of this body. Photo 8 illustrates what may be regarded as the type. Indeed, in my study of this element, as I came to examine by the hundreds division figure after division figure it became impressed upon me that this was in reality the one most constant identifiable element in the whole phase of spermatogenesis.

If this X-like body is merely accidental, then it is one of the most astonishingly consistent accidents I have ever encountered. It has a relatively constant size and shape, it ordinarily is not likely to be confused in the least with other elements present in the spermatocyte, and it is an accident which has the very same appearance in the testicular cells of Langshan, Plymouth Rock and Rhode Island Red fowls. Furthermore, a similar accident occurs in the guinea-fowl, only there it is consistently comma-shaped (Fig. 160) instead of being a curved element of uniform thickness. Moreover, in the guinea-chicken hybrid a similar element resembling more that of the guinea parent can be identified, and lastly, on the accident theory, the crowning wonder is that in all of these forms, the element in question

should imitate so consistently the behavior of an X-element. It seems to me that one must be credulous indeed to catalog it all as accidental.

When I take into account all of the evidence which I have been able to accumulate through some ten years of study on this problem, I feel more firmly convinced than ever that in this distinctive curved chromosome we are dealing with a body comparable to the so-called X-element of other forms. Certainly the evidence of this, as regards numbers of X-like chromosomes actually seen is much stronger than that which has been accepted unquestioned for certain of the less typical cases in invertebrates. However, in view of the condition which I have found existing in certain somatic and early germ-cells of the male where in a large number of instances two special curved chromosomes occur, I am strongly of the opinion that the large curved element of the primary spermatocyte is in reality these two earlier elements fused into one. It is just about the size that two such elements would have when fused; moreover, no further trace of the latter is discernible after the spermatogonial stages are passed. Then again, such a doubling would account for the relatively larger size of the curved element in comparison to the other chromosomes of the primary spermatocyte. My interpretation of the condition then, insofar as I can analyze the probabilities of the case, is that the two special curved elements of the spermatogonia fuse in the primary spermatocyte and act as a single element, typically passing undivided to one pole of the mitotic spindle and thus producing a dimorphic condition of the ensuing spermatocytes of the second order, half of which will contain the X-like element, half be without it.

Secondary Spermatocytes.

The secondary spermatocytes are of approximately the same size as the original spermatogonia. Although resting nuclei are not infrequently to be found, in many cases the chromosomes of the primary spermatocyte telophase rearrange directly to form the metaphase of the secondary spermatocyte. In so doing there is a marked tendency for the chromosomes to fuse by twos so that instead of the expected sets of eight and nine re-

spectively received from the primary spermatocyte, a smaller number, usually four (Figs. 175–183, photos 36, 37) or five (Figs. 161–163), appear at the equator of the new spindle. The groups of four I interpret as characterizing those cells which received only the eight ordinary chromosomes at the preceding division, the groups of five, those which received the extra, curved element in addition to the eight ordinary ones. This view is strengthened by the fact that in the groups of five, the fifth element (Fig. 163) may often be seen to resemble the original special element of the primary spermatocyte. Furthermore, at the time of division this element lags behind like a typical X-element and often does not divide until the other chromosomes are well on their way toward the poles of the spindle (Figs. 168, 172, 174, photo 63).

While my best preparations show groups of four and of five respectively to be by far the prevailing type, it is not unusual to find other cases in which the number may be six, or seven—any numerical combination, in fact, that can be made by the union in twos of one or more pairs of chromosomes out of a total of eight. The explanation seems to be that while pairing of the ordinary chromosomes is the rule, this union is sometimes incomplete or does not occur between certain individuals. In both the four and five groups one of the chromosomes is smaller than the others (Fig. 177) and where only one pair of the autosomes remains unpaired in the secondary spermatocyte, it seems most frequently to be the two components of this smaller bivalent one (Figs. 184 and 185). This gives five chromosomes without the X-like one in what typically is a four-group, and six chromosomes in what otherwise would have been a five-group where the curved element is present. In early metaphase of the secondary spermatocytes a bipartite condition of the chromosomes preparing to divide not infrequently reveals their double nature (Fig. 180). Moreover, the chromosomes may tend to resolve into their original univalent condition as they progress toward the poles presumably after having divided as a four or five group (Figs. 168, 172). The occasional finding of groups of eight or nine univalent chromosomes at one pole or the other in the telophase of such a division indicates that the division is in no sense a reduction division as was probably the preceding one. Fig. 164

and photo 34 show cases in which the chromosomes which have passed to the poles of the spindle in the primary division are in process of rearrangement for the secondary division, the X-like body remains with one group only. The autosomes have fused or are fusing in pairs.

In a very few instances spindles bearing the same number of chromosomes as occur in the primary spermatocytes were found. The chromosomes were relatively small as if of the univalent type. These are possibly to be interpreted as secondary spermatocytes in which the usual fusion has not occurred, but on the other hand the evidence is not clear that they are not primary spermatocytes in which the fixing agent has caused an unusual shrinkage. In any event, supposing these are in reality secondary spermatocytes, out of thousands of secondary spermatocytes examined I have found only fifteen cases of this kind. All the others, even where the exact number of chromosomes could not be determined, showed a chromosome number that was certainly less than that of the primary spermatocyte.

There has been a disposition on the part of some to question the existence of this second diminution in chromosome number. In my own work I first came across it in studying the spermatogenesis of doves and pigeons (Guyer, '00) in which forms its existence has also been confirmed by the more recent work of Geoffroy Smith ('12). Later I described it again in the guinea-fowl ('09*a*) and the chicken ('09*b*). A similar occurrence has been recorded for several mammals (*e. g.*, Jordan, '11, Wodsedalek '13). In any event, the fact remains that four-groups and five-groups are present in abundance in the testis of the common fowl. They are so obvious indeed at every turn in a good preparation that it has been a long standing puzzle to me why there has been any difficulty on the part of others in demonstrating them. They have become an object of routine demonstration in my own cytological laboratory which even beginners in the course have little difficulty in finding.

Photos 36–54 show various groups of four. Photos 47–52 represent anaphases where even in a photograph, with its limitations as to planes, four chromosomes are revealed at one or both poles. In photos 51 and 52, to show detail at one end the other

has had to be thrown out of focus but the four chromosomes can be demonstrated there just as clearly. Figs. 186–188 show more clearly the conditions which exist in these four-groups inasmuch as some depth of focus has been added. It is obvious that, as in photos 42–46, where the chromosomes are arranged around the equator of the spindle and the latter is seen from the side, only three of the chromosomes can be photographed as the fourth lies behind the middle one and in a different plane. Such photos as 37 and 41 of polar views reveal the true condition of affairs. Figs. 181–183 show the real constitution of such groups when seen from the side under the microscope where the focus can be shifted.

Photos 55–64 and Figs. 165–174 show views of five-groups. Photo 61 and Figs. 169–171 represent such a group in anaphase of division. One end is in better focus than the other in the photograph; the figure (Fig. 169) reveals the true condition which is as clear cut as if stamped with a die when examined under the microscope. Photos 63 and 64 are of five-groups in which, although the regular chromosomes have divided the X-like one has lagged at the equator of the spindle and is just in process of division. Figs. 167, 168, 172–174 show camera lucida drawings of other somewhat similar stages.

Spermatids.

Inasmuch as the secondary spermatocytes were dimorphic the condition is maintained in the spermatids, of which, therefore, there are typically two classes; namely, those which receive four chromosomes (probably eight univalent ones) and those which receive five chromosomes (probably nine univalent). In most cases, at the conclusion of the last division the chromosomes mass more or less and become a center around which the new nuclear membrane appears.

Although resting spermatids with reticular nuclei are to be seen, frequently the spermatids seem to proceed to spermiogenesis without a vegetative or resting stage; that is, the chromosomes seem to mass for the transformation without passing back into the diffuse stage.

It should be mentioned at this point that some of the spermatid

nuclei seem to proceed to further division. This anomalous division, in so far as I have been able to follow it, seems to be confined to the spermatids which have received the four chromosomes. Photo 69 shows in the upper field a telophase of a secondary spermatocyte which is dividing as a four-chromosomed cell to form two spermatids which will obviously contain four chromosomes each. Below it, however, is such a division of a four-chromosomed spermatid as I have just mentioned. The size relations of the two are obvious. Figs. 193 and 201 show similar conditions. I am inclined to believe that even further divisions of such four-groups occur inasmuch as it is not uncommon to find still smaller cells with four small chromosomes in process of division. Photos 65, 66 and Figs. 196 and 197 represent such a type. They may become so small as to make it difficult to decide whether one is dealing with 4 small chromosomes or a quadripartite chromosome. On the other hand, one may from time to time encounter division figures in which, instead of four chromosomes, only two appear at the equator of the spindle (Fig. 198, photo 68) suggesting that the fours have again paired.

Such unaccountable behavior on the part of the many four-chromosomed cells suggests that these cells have run riot, as it were, and are to be looked upon as degenerating. This condition, together with the fact that there are many cells among the spermatids which look as if they were not normal (Fig. 204) makes it appear probable that the one class of spermatids does not transform into adult spermatozoa. Moreover, careful measurements of spermatozoa from the vas deferens fail to reveal more than one type.

Fig. 204 represents forms which occur very commonly among the spermatids. Instead of an elongation of the nucleus and a rearrangement of the chromatic bodies into the form characteristic of what may be interpreted as the normal course of development (Figs. 200, 203), the chromatin compacts into a single dense round or very irregular mass. Occasionally what looks like an incipient axial filament appears but does not develop far. At least, I would so interpret the many instances which occur as represented in Fig. 204. Not infrequently also

spermatids are to be found which are indefinite in nuclear make-up and seemingly impaired in some way.

It cannot be positively affirmed, of course, that such cells as are represented in Fig. 204 are the product of the four-chromosomed class instead of the other. Such an interpretation is merely an implication which grows out of what appears to be other aberrancies in the behavior of the four-chromosomed class.

While at first glance, this view that one entire class of spermatids degenerates without becoming functional may seem improbable to some, it should be borne in mind that such an occurrence is by no means unique in spermatogenesis. In both phylloxerans and aphids, for example, just such an aborting of one of the two classes of sperm-forming cells occurs, to say nothing of the even more remarkable conditions which obtain in bees and in certain hermaphroditic forms such as *Rhabditis*.

Figs. 200 and 203 represent stages in what I interpret as the normal sequence of transformation. In such cases, by the time the axial filament first appears the nucleus has already begun to elongate slightly. Typically the chromosomes from the last division seem to arrange themselves into more or less of a closed ring around which the nuclear membrane forms. They then tend to concentrate gradually toward one side of the nucleus along the periphery until they form more or less of a crescent which thickens and shortens as the process continues (Fig. 200). The nuclear membrane, along the margin free from chromatin, fades from view as the transformation progresses, leaving finally an elongate chromatin mass rounded at one end and more sharply pointed at the other. The pointed end is seen to be provided with a definite head spine and the blunter end to be in juxtaposition to the axial filament. The latter seems to develop in the typical way from the divided centrosomes, one of which has become knoblike and the other a ring.

Instead of following what appears to be the simpler course of development as depicted in Fig. 200, the nucleus seems frequently to transform into the spermatozoön head by a process of uncoiling, representative stages of which are shown in Fig. 203. As is the case in other various types of spermiogenesis, fragments of cytoplasm appear not infrequently to be cut off from the

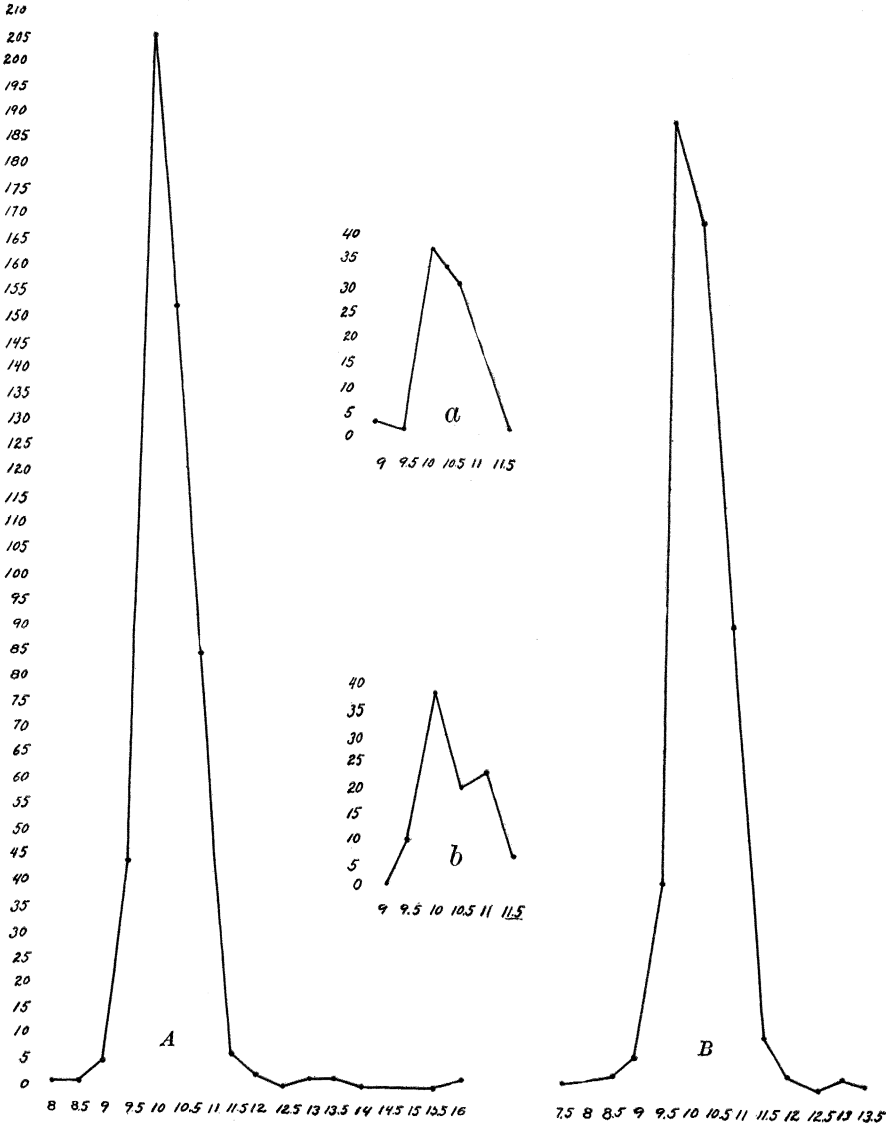
cytoplasm of the transforming spermatid and left behind. It is not easy actually to establish this, but such would be the inference based upon the many non-nucleated bits of cytoplasm scattered among the spermatids, together with the fact that lobes of cytoplasm may be seen projecting from many of the transforming cells. On the other hand it is possible that these bits are cytoplasmic fragments from degenerating spermatids.

SPERMATOZOA.

In my earlier paper I came to the conclusion that there were two classes of spermatozoa. My present work shows that I was probably in error on this point. My earlier work was based on measurements of the heads of 100 spermatozoa as they existed in smears of the testicular material itself. While no curve was plotted my older notes show that I obtained two means standing in about the relative ratios of 8 to 10. What would have made the $8\frac{1}{2}$, 9, and $9\frac{1}{2}$ groups, however, would afford but slight depression in a bi-modal curve. Furthermore, when later I came to measuring spermatozoa from the vas deferens, as I should have done for the earlier work, I found that the spermatozoa taken from testicular smears show a larger size and a far greater range of fluctuation in length of head than do those from the vas deferens. This means that doubtless many of those I measured in my earlier work had not yet completed their transformation and settled down to their final size.

The present measurements were made in four series based upon the length of head of spermatozoa taken from the vas deferens of two Plymouth Rock fowls. Two of the series, one from each fowl, including measurements of 500 and 515 spermatozoa respectively, I myself made. The other two, each consisting of measurements of 100 spermatozoa, were made as a control by an instructor (Dr. Elizabeth A. Smith), who is accustomed to doing cytological work but who was wholly in the dark as to what result had been obtained in my own measurements. The instructor worked with material from the same fowls but on different slides from the ones I used. The details are shown in Text-figure 2.

It is by no means an easy task to obtain such measurements



TEXT-FIG. 2. *A*, frequency distribution of head-lengths of 500 spermatozoa from a Plymouth Rock fowl.

Scale value	8	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15	15.5	16
Frequency	1	1	5	42	206	151	83	6	2	0	1	1	0	0	0	0	1

a, frequency distribution of head-lengths of 100 spermatozoa from the same fowl in a different preparation and by a different observer.

Scale value	9	9.5	10	10.5	11	11.5
Frequency	3	2	37	31	25	2

with the necessary degree of accuracy. It is not always easy to determine the exact base or the exact apex of the head. The most vexatious fact was that the long heads of nearly all of the spermatozoa were slightly curved and it required much searching to find ones sufficiently straight to measure accurately. Other likely sources of error were guarded against as carefully as possible. As nearly as could be done exactly the same fixation and degree of staining was secured in each slide. Care was taken to see that the film of seminal fluid was spread on in approximately the same degree of thinness and that only such parts of the preparation were studied as appeared to be wholly free from stretched or distorted spermatozoa. To eliminate the personal equation, as already related, another person was obtained to do two series of counts. Still other sources of error such as might arise through foreshortening of the object to be measured, or fatigue on the part of the observer, were guarded against.

A Leitz Stufen-Mikrometer in a No. 2 eye-piece was employed and the readings were based on measurements down to the mid-field between the individual lines. The lines, as is usual in micrometers, were laid off in tens. Inasmuch as relative sizes were all that was desired the figures accompanying the various curves represent merely divisions of the ocular micrometer. It is evident (Text-fig. 2) that three of the curves show no trace of bi-modality. A slight trace appears in *b*, but so slight as to be negligible since the curve is based on measurements of only one hundred spermatozoa and the actual differences between the 10.5 group and the 11 group is only three individuals, there being 20 spermatozoa which measured 10.5 and 23 which measured 11. Moreover *B* is a unimodal curve based on measurements of 515 spermatozoa from the same individual as that from which those measured in *b* were taken. With the differential X-like

B, frequency distribution of head-lengths of 515 spermatozoa from a second Plymouth Rock fowl.

Scale value	7.5	8	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5
Frequency	1	2	3	7	40	188	169	90	10	2	0	2	1

b, frequency distribution of head-lengths of 100 spermatozoa from the same fowl as in *B*, in a different preparation and by different observer.

Scale value	9	9.5	10	10.5	11	11.5
Frequency	1	11	38	20	23	7

element as large as it is, if one half of the spermatozoa possessed it and one half did not, one would certainly expect to obtain a fairly well marked bi-modal curve in the measurements of such spermatozoa. The presumption is therefore that only a single class of spermatozoa are represented. The probability that this class is the one developed from the five-chromosomed spermatids has already been indicated. While one wishes that the evidence might be more decisive on this point, no more significant facts seem forthcoming under present conditions of technique. What evidence there is points to the interpretation I have given and I have found no facts which indicate the contrary.

Conclusions.

Many as are the pitfalls and unsatisfactory as are parts of the evidence, I feel that I have examined a large enough number of cases and have studied a sufficient number of interpretable stages in chromosome behavior to proclaim the foregoing account as substantially the ordinary course of spermatogenesis in the common fowl. I feel that the chief hiatus centers about the fate of the one class of spermatids; that is, as to whether the degeneration evinced is confined to the four-chromosomed class, whether this class never forms spermatozoa, and whether all cases I have regarded as abnormal are really conditions of degeneration. But in the light of the fact that as a result of the transformations of spermatids only one class of spermatozoa arises I feel that only one class of spermatids completed the course of final transformation.

To those who have followed the recent literature on sex-linkage in fowls the significance of the conclusions arrived at in this paper is obvious. The facts of such linkage have been reviewed so often in recent papers and books (*e. g.*, cf. Morgan, "Heredity and Sex," 1913) that it is needless to lengthen my paper by restating them here. It is sufficient to mention that certain characteristics such as color pattern are inherited in a manner to indicate that in fowls the female is heterozygous, the male homozygous, for sex and sex-linked factors. My earlier study in which the X-like chromosome therein described was regarded as an ordinary X-element and therefore presumed to exist as

one rather than a pair of elements in the male somatic cells made the cytological evidence, if we were to continue to regard this accessory element as specifically concerned with the sex-linked characters, apparently stand at variance with the facts established by breeders. The evidence as presented in this paper, if I have correctly interpreted it, does away with this difficulty, since the female soma as seen in chick embryos is heterozygous and the male soma is homozygous for a special curved element which to my mind fulfills the requirements for being regarded as a bona fida X-element.

SUMMARY.

1. My later studies confirm my earlier ones as regards the finding of a large curved chromosome in primary spermatocytes, comparable to the so-called sex-chromosome of other forms.

2. The presence of this element is recorded in 963 primary spermatocytes which were sufficiently well prepared to show interpretable detail. It has been observed in many others. It probably exists in all although often obscured by the other chromosomes which tend to stick together.

3. It is, for variously fixed material, surprisingly constant in shape and size, in Langshan, Plymouth Rock and Rhode Island Red fowls.

4. A similarly constant element, differing in form from that of the common fowl, is found in the guinea and in the guinea-chicken hybrid.

5. At the division of the primary spermatocyte this element passes undivided to one pole thus producing two classes of secondary spermatocytes, one with nine and one with eight chromosomes.

6. The element in question is probably a bivalent chromosome formed by the union of two characteristic curved chromosomes which occur in spermatogonial and somatic cells. These elements may be seen to best advantage in the testicular or nephridial cells of 10 to 14 day chick embryos. The remaining chromosomes, typically 16 in number, are usually rod or block shaped.

7. In female chick embryos of 10 to 14 days, a relatively large percentage of dividing cells which were found in the ovarian and nephridial tissues showed a single large curved element.

8. Thus the evidence indicates that the male fowl is homozygous, the female heterozygous for this particular element.

9. The secondary spermatocytes when ready for division display, as a rule, four and five chromosomes respectively. The eight chromosomes which passed to the one secondary spermatocyte have paired to form four, and eight of the nine which passed to the other secondary spermatocyte have paired similarly, leaving the curved one unpaired.

10. The second division is regarded as not a reduction division since in the anaphase the daughter chromosomes often tend each to become bipartite or to resolve completely into two, thus revealing their dual nature.

11. Occasionally the pairing in the secondary spermatocytes is incomplete so that any number between four and nine may appear for division.

12. The odd element after lagging for some time at the equator of the spindle in the secondary spermatocyte, divides.

13. The spermatids which receive four chromosomes frequently pass on to one or more additional divisions. These are regarded as abnormal. Other evidences of degeneration in various spermatids indicate that a considerable number do not develop into normal spermatozoa. It seems probable that only one class of spermatids, that with the odd element, become spermatozoa.

14. The frequency distribution of head-lengths of spermatozoa in four different sets of measurements by two different observers shows no evidence of more than one class of spermatozoa.

15. The cytological evidence as presented in this paper harmonizes with the evidence derived from experimental breeding which shows the female to be heterozygous and the male homozygous for sex and sex-linked characters.

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EXPLANATION OF PLATES.

PLATE I.

From photographs by the author. Enlargement 1,250 diameters unless otherwise specified. Photos 1 to 7 are from sections; the remainder, from smears.

FIG. 1. Polar view, metaphase of primordial germ cell in testis of thirteen-day chick; focused to show two characteristic curved chromosomes (one at the right and the other above and to the left); the other chromosomes, mostly out of focus, are rod-like.

FIG. 2. Tangential view, metaphase of primordial germ-cell in testis of thirteen-day chick focused to show two characteristic, curved chromosomes; the other chromosomes were out of focus.

FIG. 3. Showing the two curved chromosomes in a spermatogonium of an adult Rhode Island Red fowl.

FIG. 4. Showing a single curved element (to the right) in an early germ-cell in the ovary of a ten-day chick. The other chromosomes are out of focus but careful examination showed no other curved ones among them.

FIG. 5. Polar view, metaphase in nephridial tubule of a ten-day female chick. The single curved chromosome lies well to one side (above) the other chromosomes.

FIG. 6. Side view of an equatorial plate stage in an ovarian cell of a ten-day chick. A less deeply staining, curved element was attached to one edge (left) of the plate. The photograph does not reveal the curved condition which was readily visible under the microscope.

FIG. 7. Another ovarian cell in a ten-day chick; condition practically the same as in 6.

FIG. 8. Side view of a metaphase in a primary spermatocyte of a Plymouth Rock fowl showing the special curved element well toward one pole. × 1,300.

FIG. 9. Side view of a metaphase in a primary spermatocyte of a Langshan fowl, showing the curved element near one pole.

FIG. 10. Tangential view of a metaphase in a Rhode Island Red fowl showing the curved element near one pole of the spindle.

FIG. 11. Side view of a metaphase in primary spermatocyte of a Plymouth Rock fowl showing the curved chromosome.

FIG. 12. Ditto. The special chromosome is curved toward the observer. × 1,200.

FIG. 13. Ditto. The special chromosome is curved away from the observer and hence foreshortened in the photo.

FIG. 14. Side view, metaphase in primary spermatocyte of Langshan fowl, the curved element seen tangentially.

FIG. 15. Ditto in Plymouth Rock fowl.

FIG. 16. Showing nature of the spindle in primary spermatocytes (Plymouth Rock). The special element is not visible though probably in the equatorial plate in some such condition as in Photo 32 (polar view). × 1,200.

FIG. 17. Side view, metaphase in primary spermatocyte of Langshan fowl. The fuzzy appearance of the curved chromosome in this and various other photos is due to a coating of linin-like material which, though in sufficient contrast to the chromosome as seen under the microscope, photographs dark. × 1,300.

FIG. 18. Primary spermatocyte of Plymouth Rock fowl; the special chromosome curved away from the observer (see Fig. 24 for a different view of a somewhat similar stage).

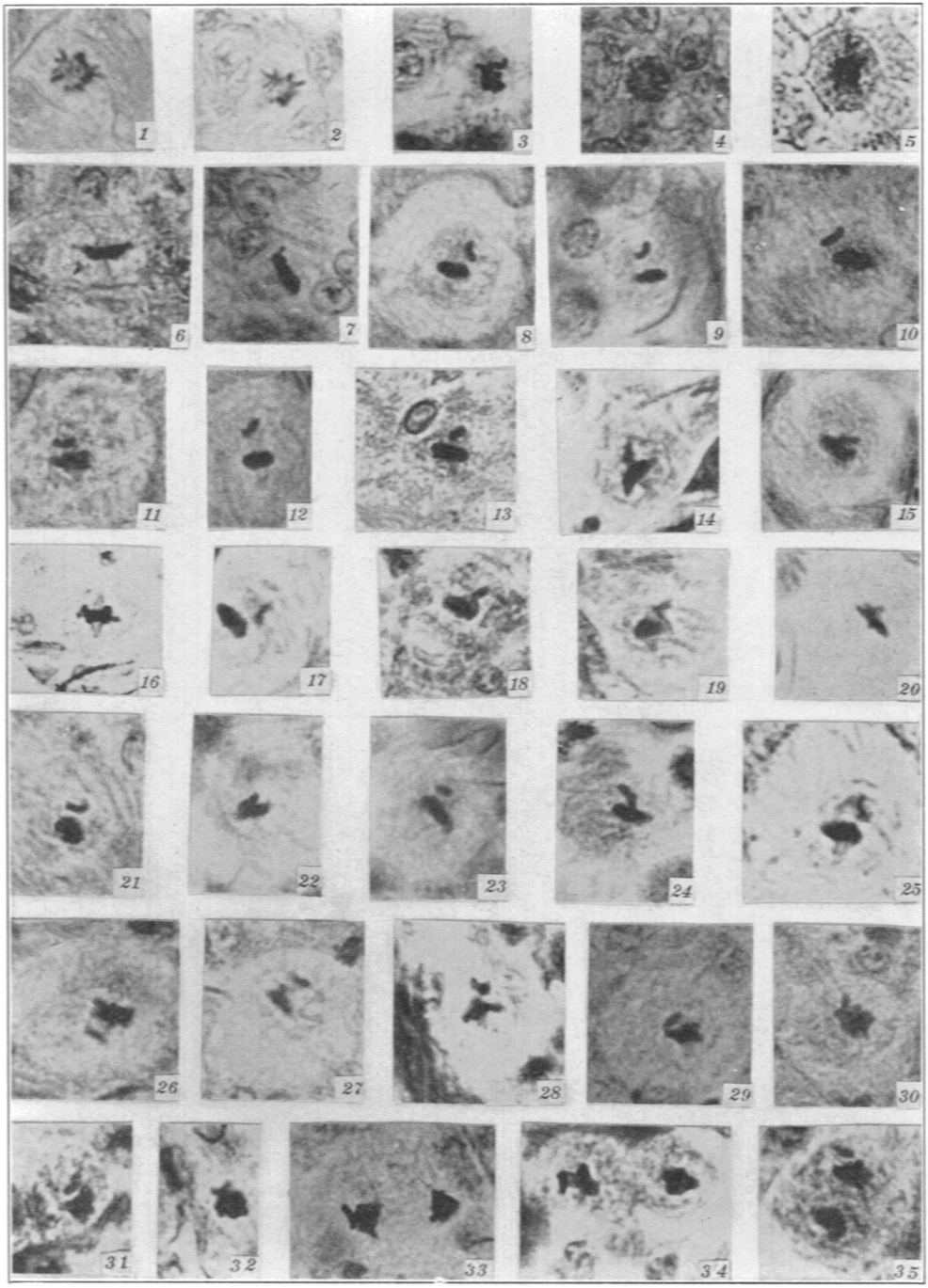

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FIG. 19. Primary spermatocyte of Langshan fowl. See comment under 17.

FIG. 20. Ditto. The curved element lies close against the other chromosomes in the equatorial plate stage and if the preparation were not greatly decolorized would be indistinguishable.

FIG. 21. The curved element in a spermatocyte of a Rhode Island Red fowl. × 1,200.

FIG. 22. Primary spermatocyte of Plymouth Rock fowl, side view of metaphase showing the curved chromosome lying just above the equatorial plate.

FIG. 23. Primary spermatocyte of Langshan fowl, the curved element seen tangentially and therefore foreshortened; easily seen to be the typical element when focus can be shifted.

FIG. 24. From Plymouth Rock fowl. See 18 for comment.

FIG. 25. From Langshan fowl. See 17 for comment. × 1,300.

FIG. 26. Ditto. × 1,300.

FIGS. 27, 28, 29. Showing various relations of the special chromosome to the spindle in primary spermatocytes.

FIG. 30. Polar view of metaphase in a Plymouth Rock fowl, the curved element lying at the upper edge.

FIGS. 31, 32. Ditto in Langshan and Rhode Island Red fowls respectively.

FIG. 33. Anaphase of a dividing primary spermatocyte in the Plymouth Rock fowl. The curved chromosome has passed over undivided to one pole.

FIGS. 34, 35. Stages somewhat similar to that shown in 33. In 34 the division has proceeded further; the cytoplasm is constricting to complete the division and the chromosomes are undergoing their second pairing, the group to the right having already formed a 4-group.

PLATE II.

From photographs by the author. Enlargement 1,250 diameters unless otherwise indicated.

All photographs in this plate were made from smears.

FIGS. 36–39. Metaphases in secondary spermatocytes of the Rhode Island Red fowl showing four chromosomes each.

FIG. 40. Side view of spindle in a secondary spermatocyte of the Plymouth Rock fowl; one of the four chromosomes has been displaced from its equatorial position, probably in making the smear.

FIG. 41. A four-chromosomed secondary spermatocyte of a Langshan fowl. × 1,200.

FIGS. 42–46. Side views of spindles bearing four chromosomes in secondary spermatocytes of the Plymouth Rock fowl.

FIGS. 47–52. Representative anaphases in the four-chromosomed type of secondary spermatocyte in Plymouth Rock (47, 49), Langshan (48, 50) and Rhode Island Red (51, 52) fowls.

FIGS. 53, 54. Equatorial plate stages in secondary spermatocytes of Langshan fowls showing four chromosomes each.

FIG. 55. Polar view of a metaphase in a secondary spermatocyte of a Plymouth Rock fowl showing five chromosomes.

FIG. 56. One end of an anaphase in a secondary spermatocyte which showed five chromosomes; two of them over lap in the photo so as to look like one.

FIG. 57. Side view of a five-chromosomed secondary spermatocyte in the Plymouth Rock fowl showing the fifth element, a curved chromosome, at one edge of the spindle. × 1,200.

FIGS. 58, 59. Stages in the Plymouth Rock similar to that shown in 57. In 58 the curved element lies at the left edge of the equatorial plate, in 59 at the right edge.

FIG. 60. Anaphase of a dividing five-chromosomed secondary spermatocyte of the Plymouth Rock fowl. × 900.

FIG. 61. Late anaphase of a dividing five-chromosomed secondary spermato cyte in a Plymouth Rock fowl. The five chromosomes at the lower pole while not distinctly visible in the photograph are just as distinct in reality as those at the upper pole. Fig. 169 is a camera lucida drawing of this cell which shows its true condition. × 1,450.

FIG. 62. Slightly different view of same preparation as shown in 61. × 950.

FIG. 63. A five-chromosomed secondary spermatocyte of the Plymouth Rock fowl, in process of division. The extra chromosome lags at the equator of the spindle until after the autosomes have divided, then it divides.

FIG. 64. A stage in the Langshan somewhat similar to 63. See Fig. 173 for a drawing of this cell.

FIG. 65. Side view of a *second* (anomalous) division of a four-chromosomed secondary spermatocyte in the Plymouth Rock fowl.

FIG. 66. Anaphase of such a cell as that pictured in 65.

FIG. 67. Side view of an equatorial plate stage in a four-chromosomed secondary spermatocyte of the guinea-fowl. × 1,150.

FIG. 68. Division figure (early anaphase) in an anomalous division of a spermatid in which two small-sized chromosomes are going to each pole. See Fig. 198.

FIG. 69. Material from Plymouth Rock fowl showing (above) an anaphase of a division of a four-chromosomed secondary spermatocyte, and also (below) an anaphase of a second (anomalous) division of such a four-chromosomed cell. × 1,300.

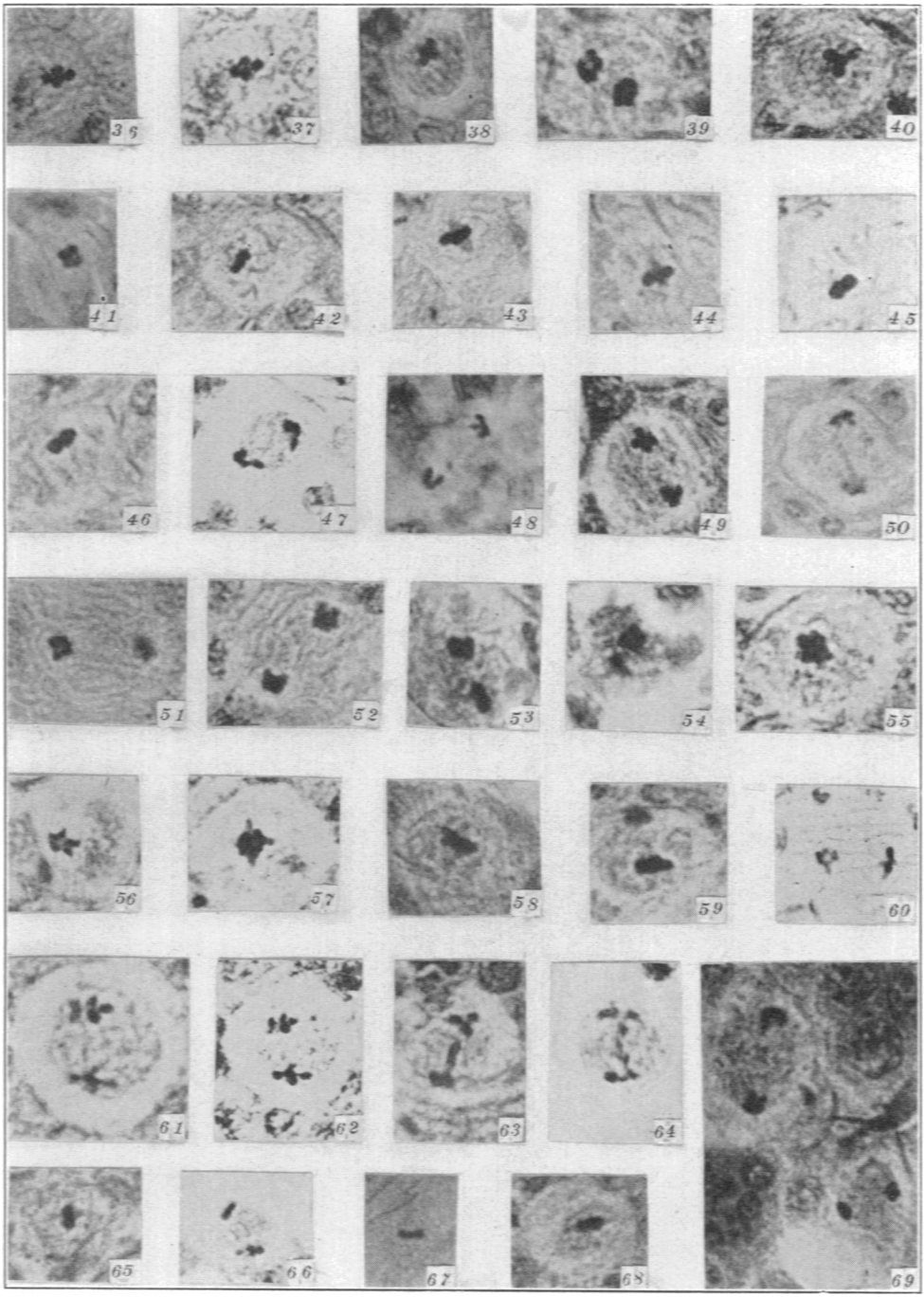

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PLATE III

All drawings on this and the following plates are the work of Miss Hattie J. Wakeman. They were made with the aid of a camera lucida. Unless otherwise specified their magnification is approximately 2,000 diameters. All on this plate are from sections of male tissues. Figs. 72, 76, 81, 86, 87 and 95 are from the testes of chick embryos. All other figures except 79 are from cells in nephridial tubules of embryos.

FIGS. 70–78. Polar views of division stages showing the two curved elements in various germinal (72, 76) and somatic cells of embryo chicks. In some a number of the autosomes have been cut away, in others all or most of the chromosomes are present. Fig. 76 is from a thirteen-day embryo, the others from ten-day embryos.

FIG. 79. Spermatogonium of adult fowl showing two curved chromosomes. The remaining chromosomal mass could not be resolved into individual elements.

FIGS. 80–85. See remarks under Figs. 70–78. Fig. 81 is from a fifteen-day embryo, the others from ten-day embryos.

FIG. 86. Side view of metaphase in primitive spermatogonial cell of ten-day chick embryo. Two relatively huge curved elements are present at each edge of the equatorial plate.

FIG. 87. The same kind of cell and the same condition as in 86.

FIGS. 88–96. Comment same as for 70–78. Figs. 88, 94, 96, from nephridial cells of ten-day embryos; Fig. 95 from testis of thirteen-day embryo.

FIG. 97. Anaphase in cell of nephridial tubule; each of the two curved elements has divided.

FIG. 98. A stage in a nephridial cell comparable to Fig. 86 of a primordial germ-cell.

FIG. 99. Probably a stage comparable to that shown in Fig. 97, only the curved elements from one side have been cut away.

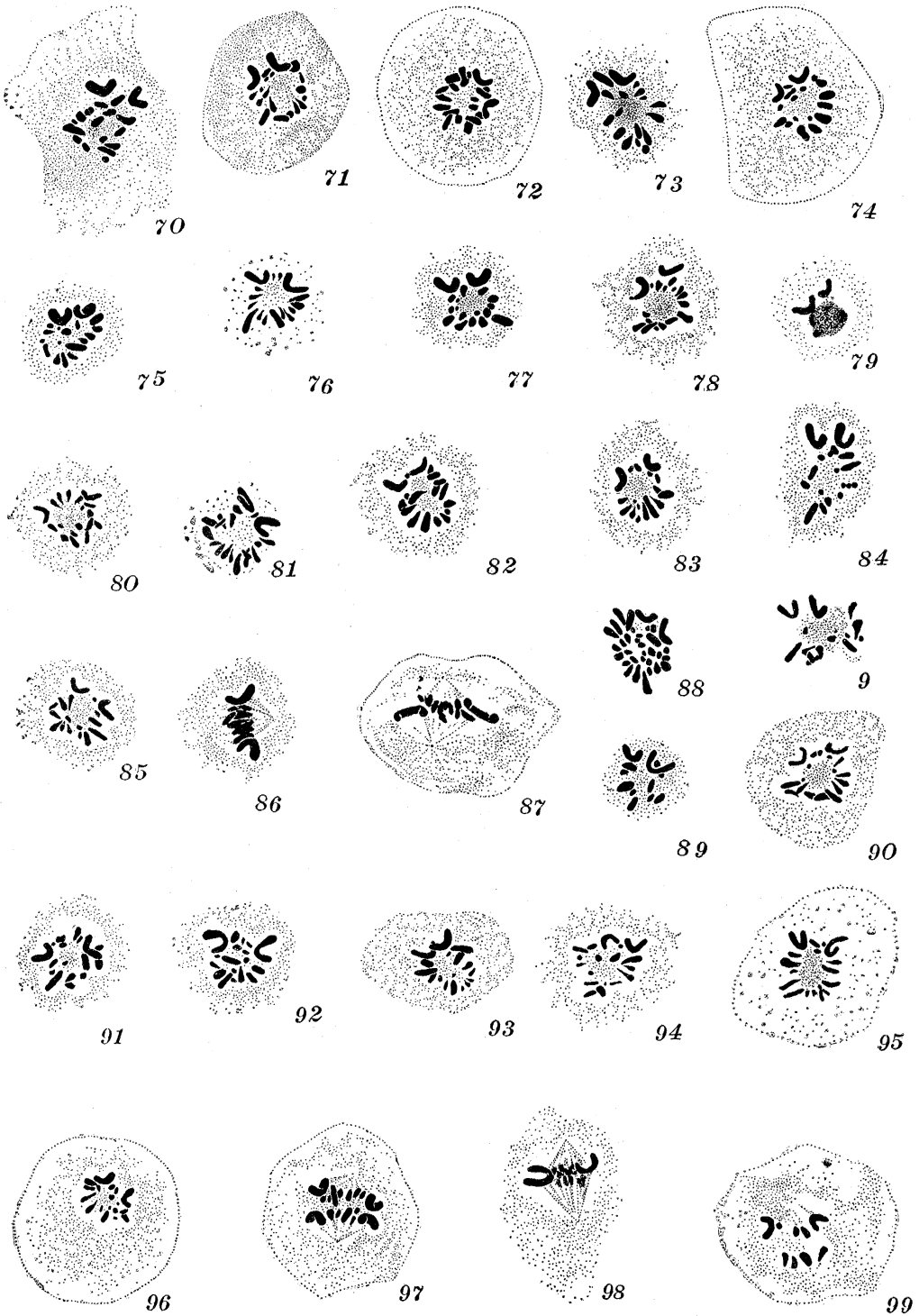
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PLATE IV.

Figs. 100 to 117 are from embryonic ovarian or female somatic tissue, from sections; 118, 119 from sections of adult testes; 120 to 132, from smears. Magnification approximately 2,000 diameters.

FIG. 100. Polar view, metaphase in primordial ovum of ten-day chick, showing a single curved chromosome.

FIG. 101, 102. Polar view, metaphases from region of gonad in five-day chick.

FIG. 103. Showing curved element in nephridial tissue of ten-day female embryo.

FIG. 104. Metaphase of a dividing cell in the nephridial tubule of a ten-day female embryo; the single curved element lies at one edge of the equatorial plate; the long axis of the spindle lies across the short axis of the cell.

FIGS. 105–108. Side views of metaphases in the germinal tissues of ten-day female embryos, each showing a single curved chromosome.

FIG. 109. Polar view, metaphase in nephridial tissue of ten-day female embryo.

FIG. 110. Division figure from germinal tissue of ten-day female embryo showing the curved element as a much lighter stained body than the autosomes.

FIG. 111. Early anaphase in a cell from the germinal tissue of ten-day female embryo showing the curved element just divided.

FIG. 112. A stage a little later than that shown in 111; from nephridial tubule of same embryo.

FIG. 113. Curved element in metaphase, nephridial tubule of thirteen-day chick.

FIGS. 114, 115, 116. Side view of division figures from ovarian tissue of thirteen-day embryo, each showing a single, long, special chromosome. In 115 the chromosome was much lighter in color than the other chromosomes.

FIG. 117. Side view of division figure in a cell from the nephridial tubule of a thirteen-day female embryo; the curved element has divided.

FIG. 118. Nuclei of spermatogonia as seen in thin sections from the testis of an adult fowl. Two elongate nucleolar-like bodies are to be seen in each. The urved nature of these bodies suggests that they may be the same as the two curved elements which appear at division time.

FIG. 119. Nucleus of late spermatogonium or early spermatocyte showing two nucleoli and general granular appearance.

FIGS. 120, 121. Nuclear phases of the early growth period of primary spermatocytes.

FIG. 122. Nucleus showing synizesis in a primary spermatocyte.

FIG. 123. Post-synizetic stage; two elongated, nucleolar-like bodies are present.

FIG. 124. Nucleus showing heavy spireme which immediately precedes the formation of chromosomes in primary spermatocytes.

FIGS. 125–132. Nuclei showing transition stages between the breaking up of the spireme and the formation of the chromosomes in primary spermatocytes. The difference in size of the nuclei is probably due to different degrees of flattening in making the smear rather than to an actual difference. Fig. 132 shows various tetrad-like groups.


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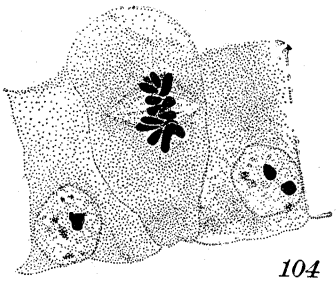
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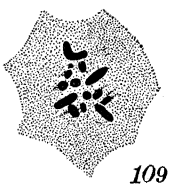
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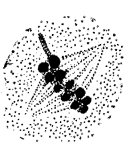
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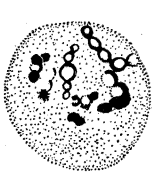
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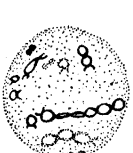
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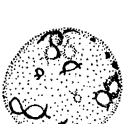
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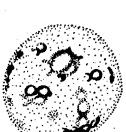
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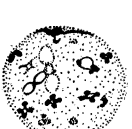
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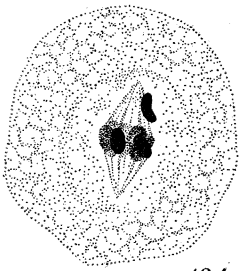
PLATE V.

Figures 137, 138, 141, 145, 148–150 are from the Langshan, Fig. 139 from the Rhode Island Red, and the others from the Plymouth Rock fowl. Magnification approximately 2,000 diameters. With the exception of 139 all the drawings are from smears.

FIGS. 134–149. Side views of division stages in primary spermatocytes showing characteristic positions and appearances of the large curved chromosome which, like a typical X-element, passes undivided to one pole of the spindle and thus gives rise to two classes of secondary spermatocytes, one with nine and one with eight chromosomes.

FIGS. 150, 151. Anaphases of divisions in primary spermatocytes showing the curved chromosome associated with but one set of the daughter autosomes.

FIG. 152. Polar view of metaphase in a primary spermatocyte showing the special curved element at one side of the chromosome mass.



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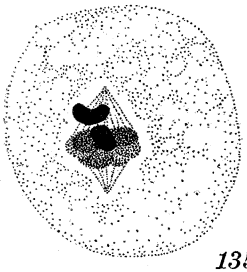

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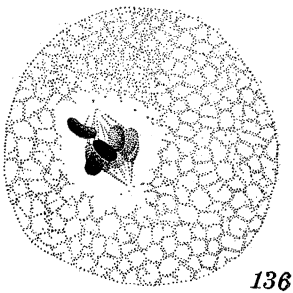

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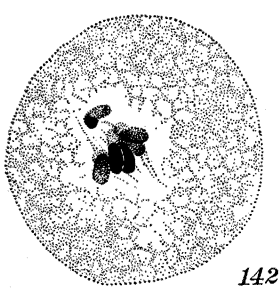

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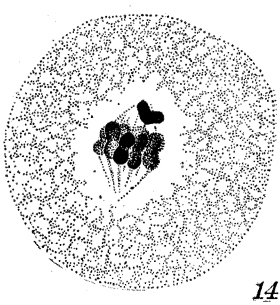

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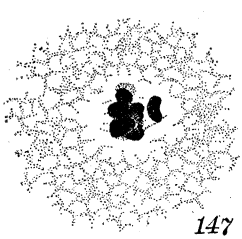

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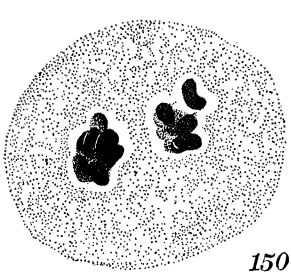

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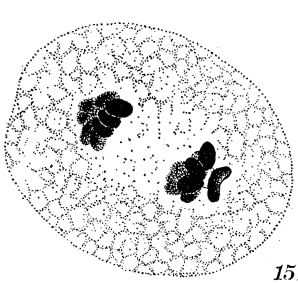

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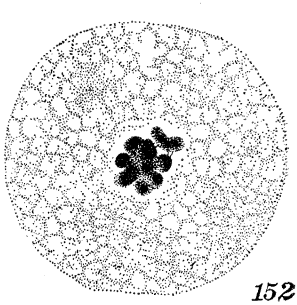

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PLATE VI.

All drawings are from smear. Figs. 168, 169, 171 are from Plymouth Rock, Figs. 153, 163–166, 170 and 174 from Langshan, and the remaining figures except 160 are from Rhode Island Red fowls. Magnification approximately 2,000 diameters.

FIGS. 153–158. Polar views of equatorial plate stages showing various relations of the special curved chromosome to the other chromosomes.

FIG. 159. Side view showing same.

FIG. 160. Polar view of an equatorial plate stage in a primary spermatocyte of the guinea-fowl. In the guinea the accessory chromosome is characteristically comma-shaped.

FIG. 161. Equatorial plate stage in a secondary spermatocyte, showing five chromosomes.

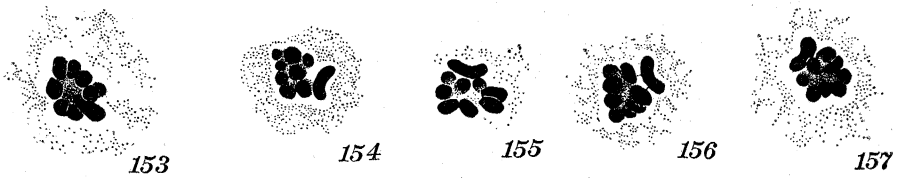
FIG. 162. A five-chromosome and a four-chromosome group lying in a common mass of cytoplasm each ready for division as a separate nucleus.

FIG. 163. Side view of an equatorial plate stage in a secondary spermatocyte, showing five chromosomes, one of which is the curved chromosome received from the primary spermatocyte.

FIG. 164. Telophase of a dividing primary spermatocyte showing secondary pairing of the daughter chromosomes in preparation for the next division. The eight ordinary chromosomes at each end pair to form four; the extra curved chromosome remains unpaired.

FIGS. 165, 166. Side views of metaphases in secondary spermatocytes showing curved chromosomes at one edge.

FIGS. 167–174. Various anaphase stages in the division of the five-chromosomed secondary spermatocytes. In 168 and 172 the double nature of the autosomes is indicated by their bipartite appearance. The lagging in division of the fifth chromosome is indicated in 167, 168, 172–174.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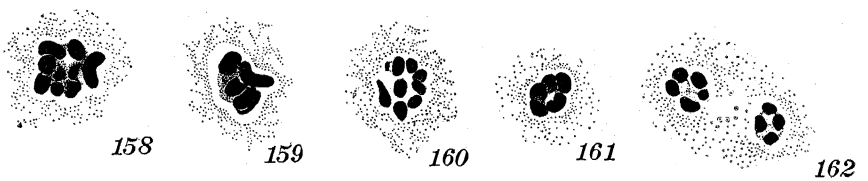
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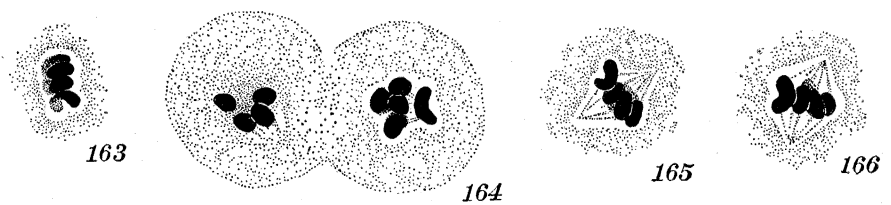
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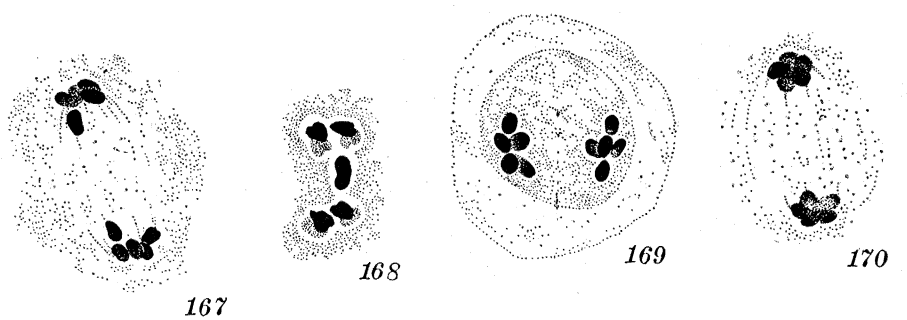
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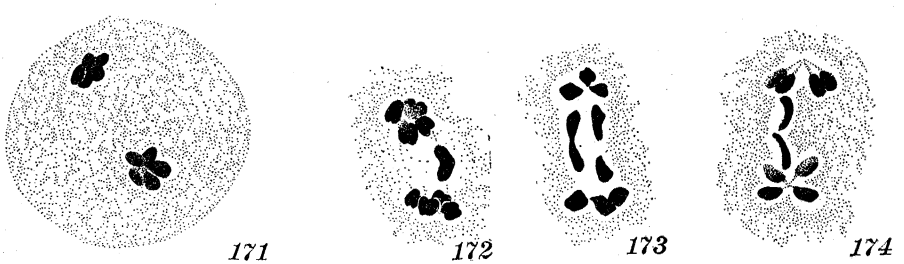

171 172 173 174

PLATE VII.

All drawings except 181, 199 and 200 were made from smears. Figs. 176, 180, 182, 183, and 186 are from the Langshan, Figs. 175, 177–179, 181, 184, 185, 187 and 188 are from the Rhode Island Red, Figs. 189, 190 from the Guinea and the remaining figures from the Plymouth Rock Fowl.

FIGS. 175–183. Characteristic metaphases of secondary spermatocytes with four chromosomes. The double nature of the individual chromosomes is shown in 180.

FIGS. 184, 185. Secondary spermatocytes showing five chromosomes. Two of the chromosomes are small and are probably comparable to one of the larger ones, having failed to unite.

FIGS. 186–188. Anaphases in secondary spermatocytes showing four chromosomes at each pole.

FIGS. 189–191. Secondary spermatocytes from the guinea fowl showing four chromosomes.

FIG. 192. Probably a second (anomalous) division in a secondary spermatocyte. Both spindle and chromosomes are reduced in size.

FIG. 193. A first and a second division of four-chromosomed secondary spermatocytes which lay side by side in a smear. The first is regarded as normal, the second is probably anomalous.

FIGS. 194–197. Various phases in the division of the smaller sized (probably anomalous) four-chromosomed cells.

FIG. 198. Anomalous division figure showing still further reduction in number and size of chromosomes than that shown in 197.

FIG. 199. Nucleus of resting spermatid.

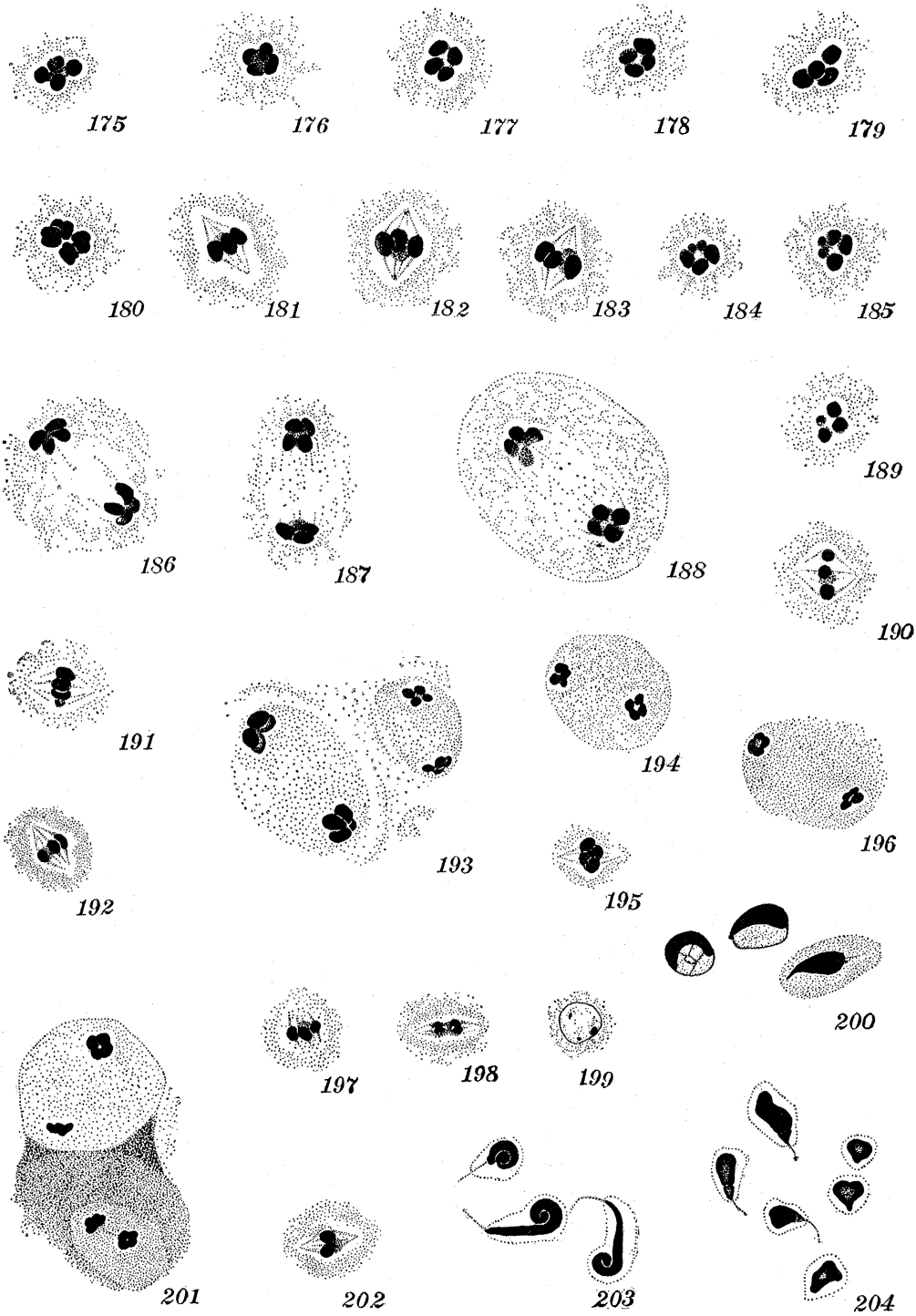
FIG. 200. Typical appearance of normally transforming spermatids.

FIG. 201. Conditions comparable to those shown in Fig. 193.

FIG. 202. A stage similar to that shown in 195.

FIG. 203. Normally transforming spermatids.

FIG. 204. Spermatids which are probably abnormal or degenerating.



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